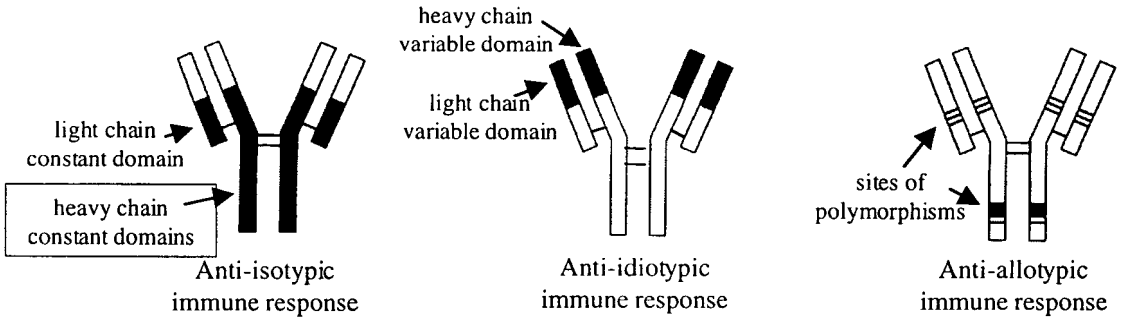
light chain
constant domain
heavy chain
constant domains
Anti-isotypic
immune response
heavy chain
variable domain
light chain
variable domain
Anti-idiotypic
immune response
sites of
polymorphisms
Anti-allotypic
immune response

Fig. 1

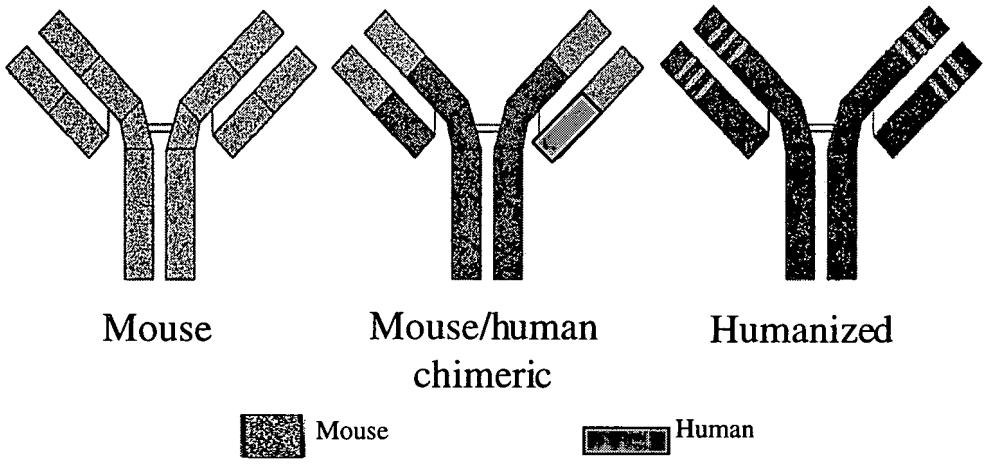
Mouse
Mouse/human
chimeric
Humanized
Mouse
Human

Fig. 2

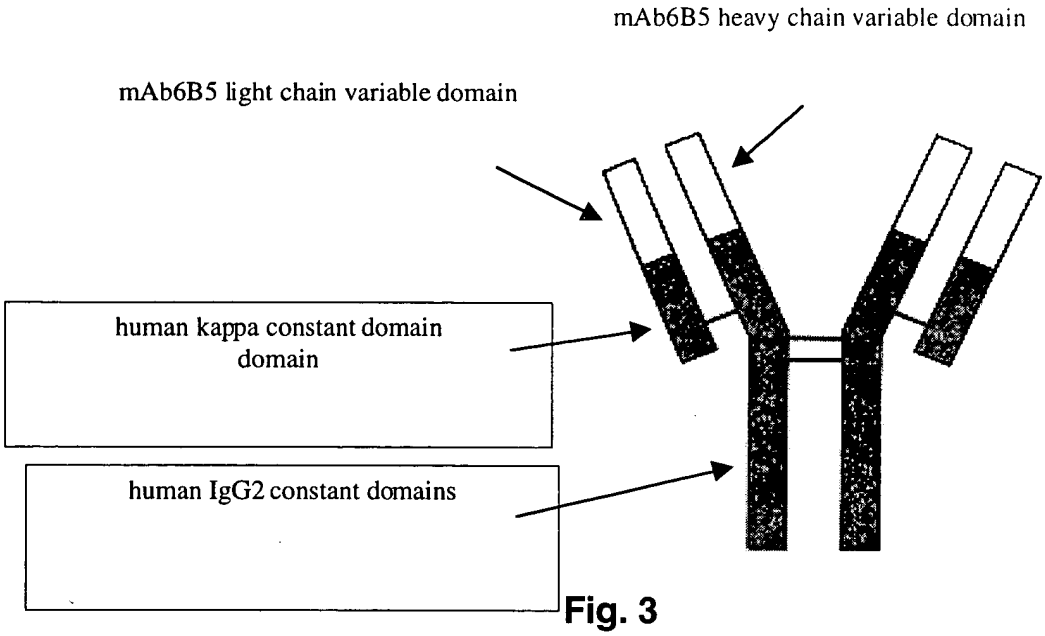
mAb6B5 heavy chain variable domain
mAb6B5 light chain variable domain
human kappa constant domain
domain
human IgG2 constant domains

Fig. 3

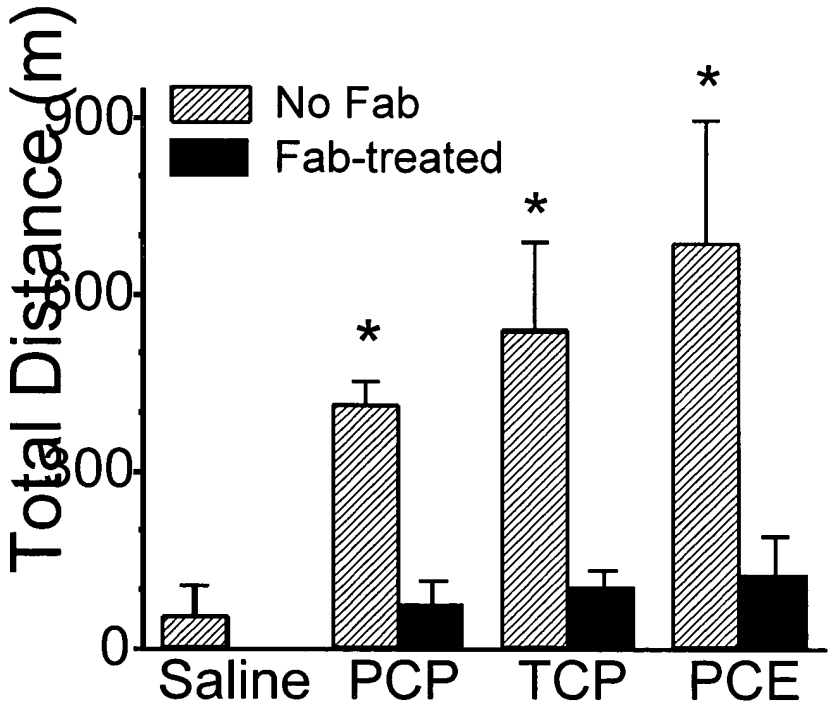
Total Distance (m)
900
600
300
0
No Fab
Fab-treated
*
*
*
Saline
PCP
TCP
PCE

Fig. 4

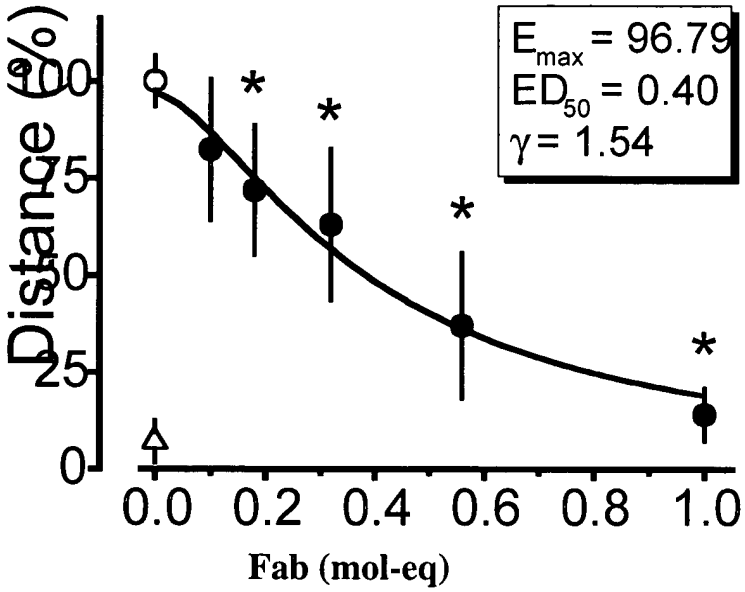
E_{max} = 96.79
ED_{50} = 0.40
γ = 1.54
Distance (%)
100
75
50
25
0
0.0
0.2
0.4
0.6
0.8
1.0
Fab (mol-eq)

Fig. 5

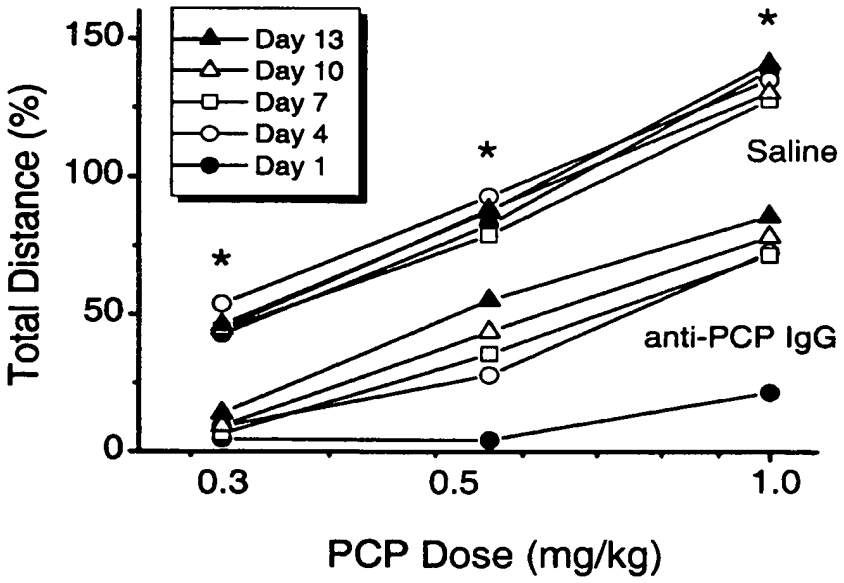
Day 13
Day 10
Day 7
Day 4
Day 1
Total Distance (%)
150
100
50
0
Saline
anti-PCP IgG
0.3
0.5
1.0
PCP Dose (mg/kg)

Fig. 6

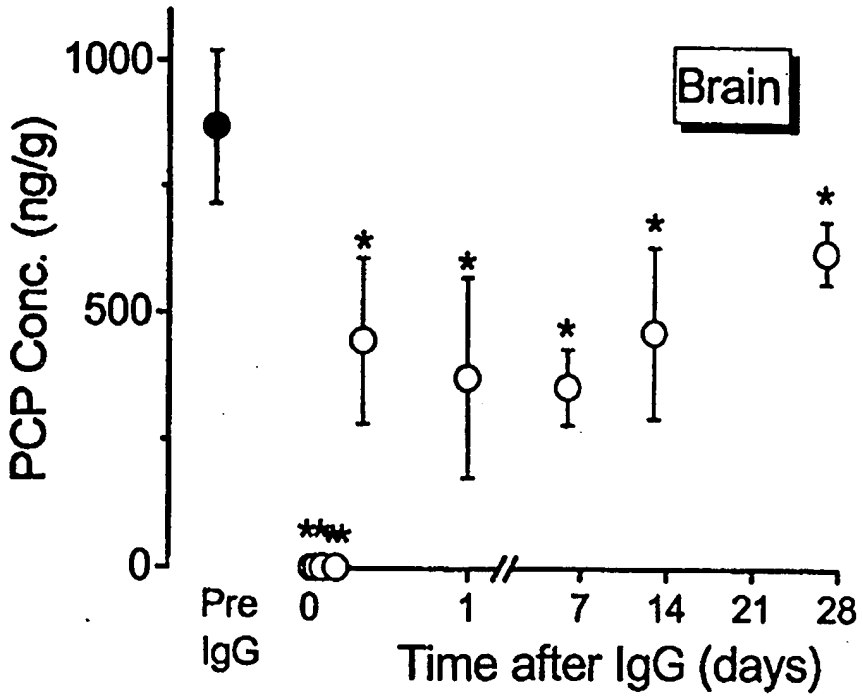
Brain
PCP Conc. (ng/g)
1000
500
0
Pre IgG
0
1
7
14
21
28
Time after IgG (days)

Fig. 7

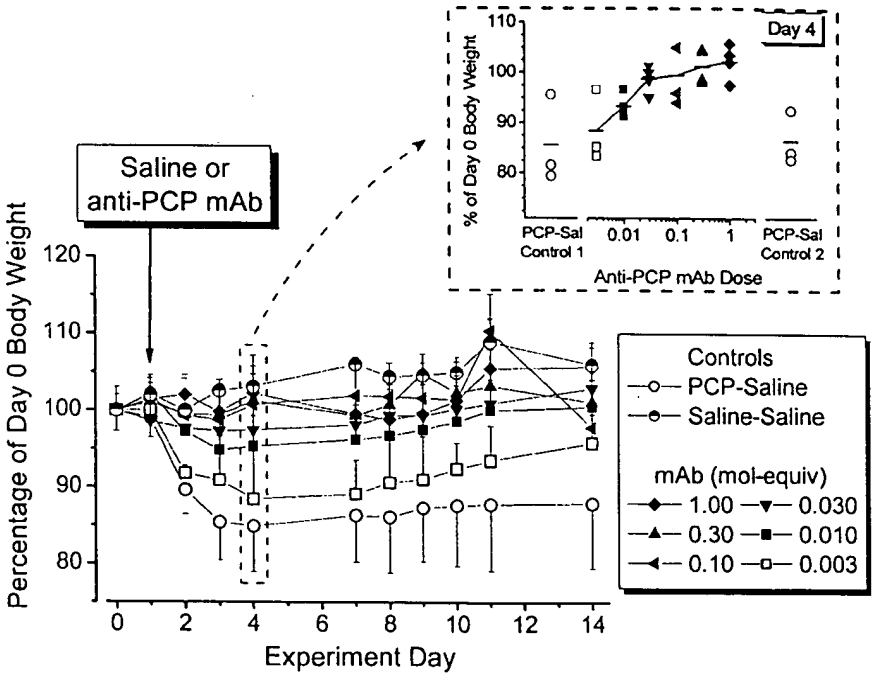
Saline or anti-PCP mAb
Percentage of Day 0 Body Weight
Experiment Day
Day 4
% of Day 0 Body Weight
PCP-Sal Control 1
0.01
0.1
1
PCP-Sal Control 2
Anti-PCP mAb Dose
Controls
PCP-Saline
Saline-Saline
mAb (mol-equiv)
1.00
0.030
0.30
0.010
0.10
0.003

Fig. 8

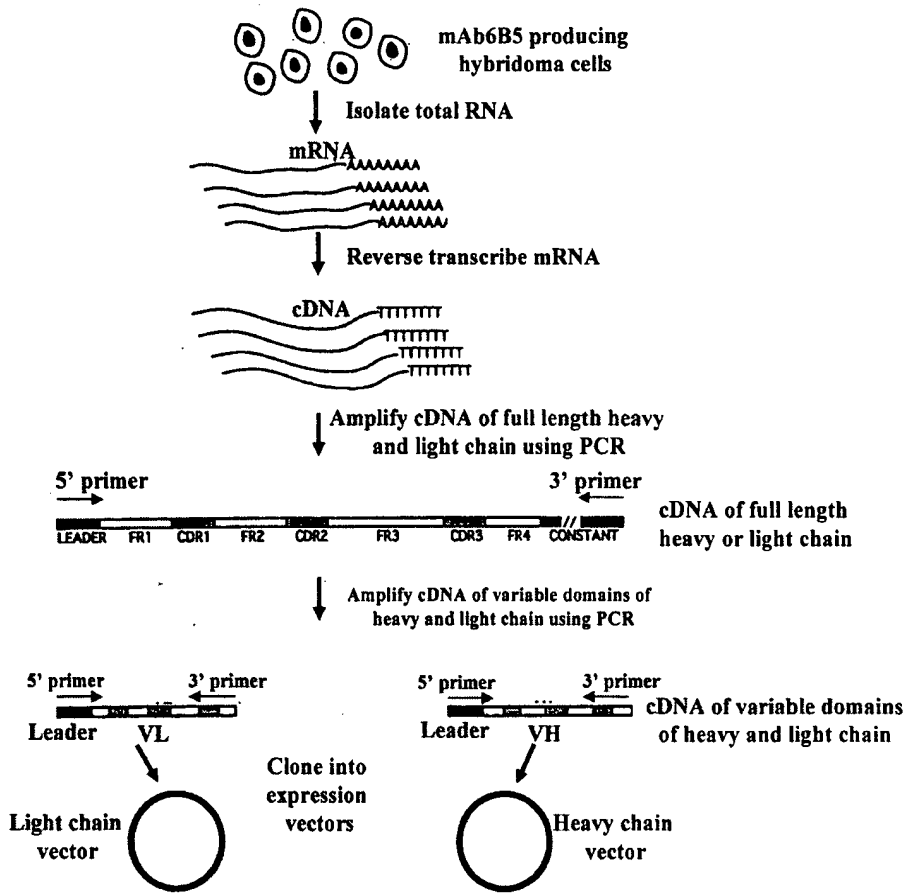
mAb6B5 producing hybridoma cells
Isolate total RNA
mRNA
Reverse transcribe mRNA
cDNA
Amplify cDNA of full length heavy and light chain using PCR
5' primer
3' primer
LEADER
FR1
CDR1
FR2
CDR2
FR3
CDR3
FR4
CONSTANT
cDNA of full length heavy or light chain
Amplify cDNA of variable domains of heavy and light chain using PCR
5' primer
3' primer
Leader
VL
Clone into expression vectors
Light chain vector
5' primer
3' primer
Leader
VH
cDNA of variable domains of heavy and light chain
Heavy chain vector

Fig. 9

```
  1 - ATGAAGTTGCCTGTTAGGCTGTTGGTGCTGATGTTCTGGATTCCTGCTTCCAGCAGTGAT - 60
    - M  K  L  P  V  R  L  L  V  L  M  F  W  I  P  A  S  S  S  D
 61 - GTTTTGATGACCCAAACTCCACTCTCCCTGCCTGTCAGTCTTGGAGATCAAGCCTCCATC - 120
    - V  L  M  T  Q  T  P  L  S  L  P  V  S  L  G  D  Q  A  S  I
121 - TCTTGCAGATCTAGTCAGACCATTGTACATAGTAATGGAAACACCTATTTAGAATGGTAC - 180
    - S  C  R  S  S  Q  T  I  V  H  S  N  G  N  T  Y  L  E  W  Y
181 - CTGCAGAAACCAGGCCAGTCTCCAAAGCTCCTGATCTACAAAGTTTCCAACCGATTTTCT - 240
    - L  Q  K  P  G  Q  S  P  K  L  L  I  Y  K  V  S  N  R  F  S
241 - GGGGTCCCAGACAGGTTCAGTGGCAGTGGATCAGGGACAGATTTCACACTCAAGATCAGC - 300
    - G  V  P  D  R  F  S  G  S  G  S  G  T  D  F  T  L  K  I  S
301 - AGAGTGGAGGCTGAGGATCTGGGAGTTTATTACTGCTTTCAAGGCACACATGCTCCGTAC - 360
    - R  V  E  A  E  D  L  G  V  Y  Y  C  F  Q  G  T  H  A  P  Y
361 - ACGTTCGGAGGGGGGACCAAGCTGGAAATAAAAACTGCGGCCGCACCATCTGTCTTCATC - 420
    - T  F  G  G  G  T  K  L  E  I  K  T  A  A  A  P  S  V  F  I
421 - TTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAAT - 480
    - F  P  P  S  D  E  Q  L  K  S  G  T  A  S  V  V  C  L  L  N
481 - AACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGT - 540
    - N  F  Y  P  R  E  A  K  V  Q  W  K  V  D  N  A  L  Q  S  G
541 - AACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGC - 600
    - N  S  Q  E  S  V  T  E  Q  D  S  K  D  S  T  Y  S  L  S  S
601 - ACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACC - 660
    - T  L  T  L  S  K  A  D  Y  E  K  H  K  V  Y  A  C  E  V  T
661 - CATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTGA (SEQ ID NO: 15)
    - H  Q  G  L  S  S  P  V  T  K  S  F  N  R  G  E  C  *  (SEQ ID NO: 16)
```

Murine 6B5 variable region leader sequence
Human kappa constant region NotI restriction site **TGA** stop codon

Fig. 10

```
  1-ATGGAATGCAGCTGTGTAATGCTCTTCCTCCTGTCAGGAACTGCAGGTGTCCTCTCTGAG-60
    -M  E  C  S  C  V  M  L  F  L  L  S  G  T  A  G  V  L  S  E
 61-GTCCAGCTGCAACAGTCTGGACCTGAGTTGGTGAAGCCTGGGGCTTCAGTGAAGATGTCC-120
    -V  Q  L  Q  Q  S  G  P  E  L  V  K  P  G  A  S  V  K  M  S
121-TGCAAGGCTTCTGGCTACACTTTCACTGACTACTACATACACTGGATGAAGCAGAGCCAT-180
    -C  K  A  S  G  Y  T  G  T  D  Y  Y  I  H  W  M  K  Q  S  H
181-GGAAAGAGCCTTGAGTGGATTGGATATATTTATCCTAACAACGGTGGTAATGGCTACAAC-240
    -G  K  S  L  E  W  I  G  Y  I  Y  P  N  N  G  G  N  G  Y  N
241-CAGAAGTTCAAGGGCAAGGCCACATTGACTGTAGACAAGTCCTCCAGCACAGCCTACATG-300
    -Q  K  F  K  G  K  A  T  L  T  V  D  K  S  S  S  T  A  Y  M
301-GAGCTCCGCACCCTGACATCTGAGGACTCTGCAGTCTATTACTGTGGAAGATCTACCTGG-360
    -E  L  R  T  L  T  S  E  D  S  A  V  Y  Y  C  G  R  S  T  W
361-GACGACTTTGACTACTGGGGCCAAGGCACCACTCTCACAGTCTCCTCAGCTAGCACCAAG-420
    -D  D  F  D  Y  W  G  Q  G  T  T  L  T  V  S  S  A  S  T  K
421-GGCCCATCGGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCGGCC-480
    -G  P  S  V  F  P  L  A  P  C  S  R  S  T  S  E  S  T  A  A
481-CTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGC-540
    -L  G  C  L  V  K  D  Y  F  P  E  P  V  T  V  S  W  N  S  G
541-GCTCTGACCAGCGGCGTGCACACCTTCCCAGCTGTCCTACAGTCCTCAGGACTCTACTCC-600
    -A  L  T  S  G  V  H  T  F  P  A  V  L  Q  S  S  G  L  Y  S
601-CTCAGCAGCGTGGTGACCGTGCCCTCCAGCAACTTCGGCACCCAGACCTACACCTGCAAC-660
    -L  S  S  V  V  T  V  P  S  S  N  F  G  T  Q  T  Y  T  C  N
661-GTAGATCACAAGCCCAGCAACACCAAGGTGGACAAGACAGTTGAGCGCAAATGTTGTGTC-720
    -V  D  H  K  P  S  N  T  K  V  D  K  T  V  E  R  K  C  C  V
721-GAGTGCCCACCGTGCCCAGCACCACCTGTGGCAGGACCGTCAGTCTTCCTCTTCCCCCCA-780
    -E  C  P  P  C  P  A  P  P  V  A  G  P  S  V  F  L  F  P  P
```

Fig. 11A

781-AAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACGTGCGTGGTGGTGGAC-840
-K P K D T L M I S R T P E V T C V V V D
841-GTGAGCCACGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCAT-900
-V S H E D P E V Q F N W Y V D G V E V H
901-AATGCCAAGACAAAGCCACGGGAGGAGCAGTTCAACAGCACGTTCCGTGTGGTCAGCGTC-960
-N A K T K P R E E Q F N S T F R V V S V
961-CTCACCGTTGTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCTCCAAC-1020
-L T V V H Q D W L N G K E Y K C K V S N
1021-AAAGGCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAACCAAAGGGCAGCCCCGAGAA-1080
-K G L P A P I E K T I S K T K G Q P R E
1081-CCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTG-1140
-P Q V Y T L P P S R E E M T K N Q V S L
1141-ACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGG-1200
-T C L V K G F Y P S D I A V E W E S N G
1201-CAGCCGGAGAACAACTACAAGACCACACCTCCCATGCTGGACTCCGACGGCTCCTTCTTC-1260
-Q P E N N Y K T T P P M L D S D G S F F
1261-CTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGC-1320
-L Y S K L T V D K S R W Q Q G N V F S C
1321-TCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCG-1380
-S V M H E A L H N H Y T Q K S L S L S P
1381-GGTAAA**TGA** (SEQ ID NO: 17)
-G K * (SEQ ID NO: 18)

Murine 6B5 variable region leader sequence
Human IgG2 constant regions NheI restriction site **TGA** stop codon

Fig. 11B

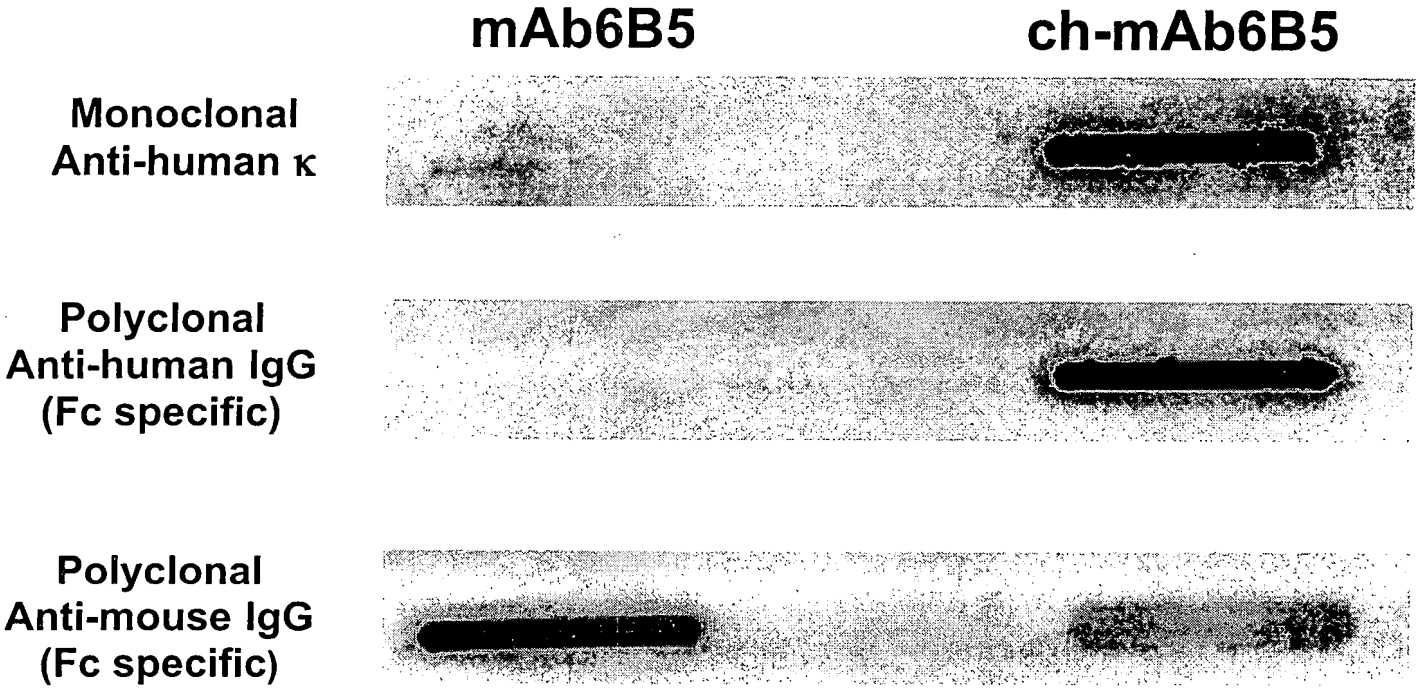
mAb6B5
ch-mAb6B5
Monoclonal
Anti-human κ
Polyclonal
Anti-human IgG
(Fc specific)
Polyclonal
Anti-mouse IgG
(Fc specific)

Fig. 12

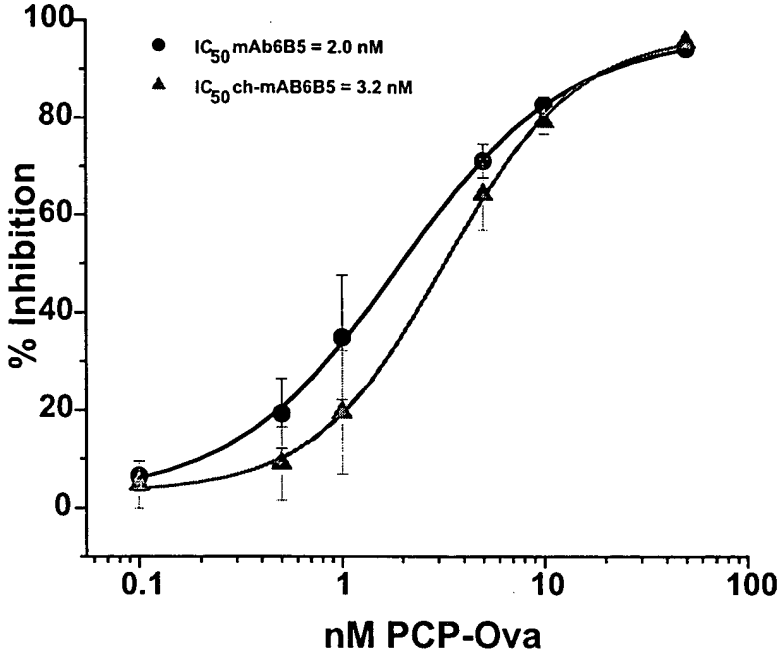
100
80
60
40
20
0
% Inhibition
IC50 mAb6B5 = 2.0 nM
IC50 ch-mAB6B5 = 3.2 nM
0.1
1
10
100
nM PCP-Ova

Fig. 13

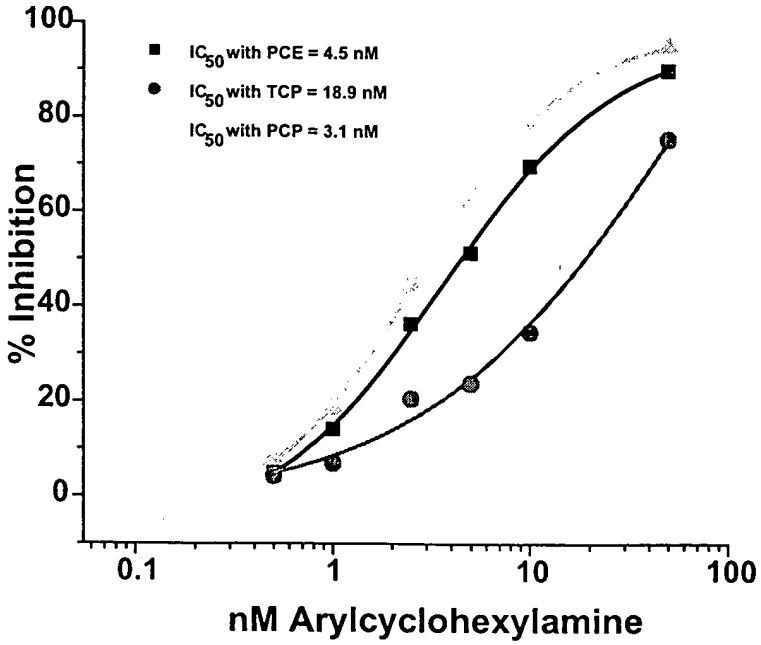
100
80
60
40
20
0
% Inhibition
IC50 with PCE = 4.5 nM
IC50 with TCP = 18.9 nM
IC50 with PCP = 3.1 nM
0.1
1
10
100
nM Arylcyclohexylamine

Fig. 14